

Perspectives of molecular marker-assisted breeding in mungbean (*Vigna radiata* L.) to develop resistance for mungbean yellow mosaic virus (MYMV) disease

Samara Mukhtar¹ and Ebtihal Alsadig Ahmed Mohamad²

¹Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad-38040, Pakistan;

²Agricultural Research Corporation, Alamin Alkarib street, Khartoum North, Sudan.

Corresponding author's e-mail: Samarachaudhry5@gmail.com

Mungbean (*Vigna radiata* L.) is a fast-growing warm seasonal pulse crop that has high nutritional and economical importance. It is grown for its seeds that have high protein content. It belongs to the family *Fabaceae*, widely distributed throughout the world mainly in Asian countries. In Pakistan, about 88% of mungbean production has occurred in the Punjab province. Various biotic and abiotic factors are responsible for a significant yield and productivity reduction in mungbean crop. While the major loss of mungbean production is mainly due to mungbean yellow mosaic virus (MYMV) which may lead to 5-100% yield loss. This disease is caused by geminiviruses, belong to begomovirus genus. To overcome this loss due to MYMV, host resistance is preferred over cultural and chemical control methods. This review summarizes the importance of begomoviruses to cause the disease, the role of vector whiteflies, implementation of various management strategies including host resistance and marker-assisted breeding. Molecular marker-assisted breeding for resistance or tolerance in mungbean against MYMV is an effective approach to combat this disease. Different molecular markers have been used to identify and characterize the mungbean resistance genotypes against MYMV. Several molecular markers have been identified through Bulk Segregant Analysis (BSA) which are linked to this disease.

Keywords: Mungbean, MYMV, begomovirus, whiteflies, marker-assisted breeding, BSA.

INTRODUCTION

Beans or pulses are considered as the source of human and animal diets having high nutritional profile. Beans are the edible legume plants that belong to family Leguminosae, also known as *Fabaceae*. Legumes are potential contributors to human food as they are rich source of nutrients. These nutrients are either macronutrients like carbohydrates, proteins, fats, dietary fibers and phytochemicals or micronutrients like minerals and vitamins. The food legumes, including the grain legumes and pulses, are the essential foodstuffs in subtropical and tropical regions of the world, where they are considered as the second most important crop after the cereals (Sreerama *et al.*, 2012).

Cereals that belong to family Poaceae include oats, wheat, rice, maize, sorghum, barley, etc. Cereal based diet has sufficient source of carbohydrates but not enough sources of proteins. About 800 million people are affected by malnutrition. So, beans are the primary source of proteins in the human diet as a food crop to meet this nutrition demand (Broughton *et al.*, 2003). Legume grains and dry seeds have

high nutritional importance for humans as well for animals. They are excellent source of proteins for the people of underdeveloped countries where there is less availability of animal proteins. Hence, pulses are commonly known as poor man's meat (Harouna *et al.*, 2018).

Legumes are whole plant or are fruits and pods of these specific plants. These legume crops are edible due to their high nutritional importance (McCrary *et al.*, 2010). All the pulses belong to legumes whereas all the legumes are not considered as pulses. Pulses such as dry beans, lentils, peas and chickpeas are harvested only to obtain dry seeds of the leguminous crops. Pulses are easily distinguishable from oil seeds (peanut and soybean) and have lower lipid content as compared to oil seeds (Tiware *et al.*, 2011). The legume crops are cultivated in adverse arid and semi-arid areas and are highly resistant to insect pests and other stresses (Bravo *et al.*, 1999). Legumes are staple of diet for all humans around the globe. They are good source of proteins (rich in lysine), complex carbohydrates such as (dietary fibers, resistant starch and oligosaccharides), antioxidants, phytochemicals, minerals such as (potassium and magnesium), vitamins such

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as (tocopherols, folates, B vitamins and ascorbic acid) and several other polyphenols (Chen *et al.*, 2010; Tiwari *et al.*, 2011; Boschini and Arnoldi, 2011; Carvalho *et al.*, 2011). Gabrelibanos *et al.* (2013) reported that legumes are not complete source of proteins just like meat and deficient in cysteine, cystine and methionine amino acids except soybean but when they are consumed in combination with other compatible cereals, they are rich in amino acids. The biological importance and the use of legumes in feed and food have been limited due to the presence of anti-nutritional components. These components are phytates, cyanogens, and phenolics, RFOs (raffinose family oligosaccharides) mainly α -galactosides, lectins (phytohemagglutinins), tannins and enzyme inhibitors (α -amylase, trypsin and chymotrypsin). Hence, due to the presence of these antinutritional factors, the bioavailability of nutrients and protein digestibility can be significantly reduced (Kalogeropoulos *et al.*, 2010; Stoddard *et al.*, 2010; Nagabhushana and Shrivastava, 2011). Xu and Chang, (2008) reported that these antinutritional components can be inactivated through various food processing ways for better taste and flavor of legume foods also, enhancing the minerals bioavailability.

Apart from their nutritional importance, legumes play a vital role in preventing the risk of some chronic diseases including cancer diseases, cardiovascular diseases, dyslipidemia (Ha *et al.*, 2014), obesity or overweight (Kim *et al.*, 2016) and diabetes mellitus (Venter and Eyssen, 2001). Recent studies indicated that by consuming the pulses and legume foods, the chances of these diseases could be reduced maximally. Legume crops are beneficial in the treatment of these disease conditions (Xu and Chang, 2008; Kalogeropoulos *et al.*, 2010; Carvalho *et al.*, 2011). As leguminous crops are rich in fiber and have low glycemic index and fat content that's why they prevent the development of diabetes. The diets with more glycemic index like potatoes and white rice can increase the blood glucose level. So, there is more secretion of insulin from pancreas and the risk of type 2 diabetes development enhances. Thus, diets with low glycemic index such as legumes and pulses prolong the return of hunger, decrease the blood glucose level and insulin secretion (Anderson, 2004).

Boschini and Arnoldi, (2011) reported that the phytochemicals like tocopherols and carotenoids are present in legume plants that may helped in limiting the chances of cardiovascular disease. The major factors that are responsible for heart disease are blood cholesterol, platelet aggregation and stress inducing endothelial dysfunction. The phytochemicals in legumes may interact with these factors and prevent their activity (Wang *et al.*, 2011). The rate of cardiovascular diseases can also be reduced by consuming diet with low glycemic index (Anderson, 2004). The polyphenolic substances such as isoflavones, tannins, lignins, flavonoids and phenolic acids are present in legumes, accounting for the anti-cancerous properties (Kolonel *et al.*, 2000). It has been studied that phytate compound shows the anti-cancerous

effect also exhibiting the antioxidant properties and effective against breast and colon cancer. The key factors of cancer are proteases and their effect may counteract by protease inhibitors and can help to combat the breast cancer (Scarafoni *et al.*, 2007).

By consuming the legume crops, pulses and many vegetables in replace of animal protein can decrease the chances of osteoporosis and excretion of calcium through urine (Venter and Eyssen, 2001). For the better bone growth during young and old age, proteins and calcium rich diets are essential. Both of these are present in higher quantity in legume foods (Rizzoli, 2008). Dietary fiber is an important component of legume foods that is important against gastrointestinal diseases and other associated diseases. These fibers can also maintain the health of intestinal system, help to fight against intestinal and colon cancer (Britannica, 2011). Three important subgenera are *Plectotropis*, *Ceratotropis* and *Vigna* whereas the subgenus *Ceratotropis* include Asian *Vigna*, and the subgenus *Vigna* is known as African *Vigna* which include several important legume crops such as cowpeas, mungbean and black gram (Pandiyan *et al.*, 2012). Beans belong to the *Vigna* genus has flowering plants in the *Fabaceae* family. The genus *Vigna* accounts for approximately 200 species having pantropical distribution, native to tropical regions worldwide. Several species of *Vigna* genus are considered as potential food crops for millions of people in developing countries. Many *Vigna* species like mungbean (*Vigna radiata*), cowpea (*Vigna unguiculata*), mat bean (*Vigna aconitifolia*), black gram (*Vigna mungo*), rice bean (*Vigna umbellata*), bambara groundnuts (*Vigna subterranea*) and adzuki bean (*Vigna angularis*) are key food staples as standard diet. These *Vigna* species exhibit considerable economic importance in recent decades (Somta *et al.*, 2007; Fery *et al.*, 2002).

The species of genus *Vigna* thrives well on harsh environmental conditions such as nutrient-poor soil, high temperature, salinity, and low rainfall. The products of beans are tender shoot tips and leaves, consumed during the seedling stage while immature seeds and immature pods, consumed during the fruiting stage. Dry seeds of beans are easy to transport and store. Some *Vigna* species are used as ground cover; forage for farm animals and green manure crops and many *Vigna* species produce edible products. Beans can be used as bean sprouts, bean paste, flour from its seeds, or as a whole bean. The overall annual production of various edible *Vigna* species approaches about 20 million hectares worldwide but most of these productions come from the developing countries (Fery *et al.*, 2002). Apart from the *V. reflexopilosa* (creole bean), most of the *Vigna* species have diploid chromosome number ($2n=2x=22$) and the genome size is relatively small (Tomooka *et al.*, 2014). Among all *Vigna* species, *Vigna radiata* alone has a major contribution to the production of a protein-based diet for societies of developing and under-developing countries (Somta *et al.*, 2009).

Mungbean (*Vigna radiata*) is a short duration (75 to 90 days) summer season food legume and can be grown as a sole crop or plays a significant role in rotation with several other crops like rice and wheat. This legume crop acquires more attention due to its fast-growing ability, easily adjusted to other cropping seasons, and thrives well on semiarid and adverse arid conditions as it requires a very little rainfall, resistance to drought and having nitrogen fixing ability (Dahiya *et al.*, 2015; Sai *et al.*, 2017). Mungbean plants are vertically straight, grows up to 90 centimeters in height in warmer temperature of about 35°C and it is highly branched legume crop that consists of clumpy pods, in the apex region of the plant, bearing 8 to 15 seed grains in them. The color of the seed can be green or brown with glabrous shape (Dhaiya *et al.*, 2015; Ganesan and Xu, 2018). Mungbean is a warm seasonal legume crop that belongs to family *Fabaceae* (Shaheen *et al.*, 2012). Mungbean is also known as green gram, moong, mash, chop suey bean, chicksaw pea, chickasano pea, chiroko, and golden gram (Lambrides and Godwin, 2007). Mungbean, in the legume family, closely resembles cowpea and adzuki bean. Mungbean plants are branched and have trifoliate leaves. The growth of mungbean can be either upright or vine type with a plant height range from about one to five feet. Mungbean plant has clusters of pale-yellow colored flowers and brown fuzzy pods. The color of mature pods can vary from yellow to brown and then black. The pod size is five inches in length having ten to fifteen seeds. The shape of the seed can be round or oblong. The color of mature seeds may vary depending upon the mungbean varieties. It can be green, brown, yellow or mottled black (Watt *et al.*, 1977).

The color of seed grains of mungbean can be helpful for consumers as its color is considered as a quality indicator. The color of cotyledons of grain seed is mostly pale yellow. Mungbean grain color is due to the tasta color. Mungbean grain has a wide range of colors from deep green, dark green, shining green, light green dull green, golden yellow, light yellow to mottled yellow (Yousif *et al.*, 2003; Katiyar *et al.*, 2007). Mungbean seed grains are characterized into five major groups depending upon its color: dull green, green with shiny seed, yellow with dull and glossy luster, brown with dull and luster seed and black with shiny luster. Almost 49% of mungbean with shiny green varieties are present in Pakistan, India, Thailand, Afghanistan, Vietnam, and the Philippines. Mungbean with dull green seed varieties is mainly present in Korea, Turkey, China, Indonesia, and Taiwan. India, Thailand, Korea, Taiwan, the Philippines, and Indonesia mostly produce varieties with yellow-colored seeds. Approximately 4% of brown varieties come from Pakistan, Iran, Afghanistan, and Iraq (Tomooka *et al.*, 1991). Mungbean can differ in its physical properties like length, width, thickness, and diameter based on its different varieties. The average length, thickness, width, and diameter of mungbean is 4.9mm, 3.6mm, 3.7mm, and 4.3mm respectively

(Nimkar and Chattopadhyay, 2001; Mangaraj *et al.*, 2005; Yildiz, 2005; Unal *et al.*, 2008). Other physical properties including the average weight of thousand seeds, volume, true density, bulk density, and porosity are 35.6g, 33.2mm³, 756.81kgm³, 1335.4kgm³, and 40.8% respectively (Nimkar and Chattopadhyay, 2001; Unal *et al.*, 2008). Mungbean plays an important role in the nutritional diet of societies. The chemical composition of mungbean includes different chemical constituents that are macronutrients, micronutrients, phytonutrients, and electrolytes. Macronutrients include carbohydrates, lipids, fatty acids, proteins, amino acids, ash, and fibers having high molecular weight. Micronutrients having low molecular weight are essential minerals and vitamins. Minerals in mungbean are comprised of copper, iron, phosphorus, manganese, zinc, selenium, and calcium. While vitamins in mungbean include vitamin-A, vitamin-C, folates, pyridoxine, thiamin, riboflavin, and niacin. Other chemical components are electrolytes and phytonutrients that consist of sodium, potassium, and carotene-β respectively (Dahiya *et al.*, 2015).

The protein content in Mungbean is higher (24% to 28.0%) than any other legume crop. While moisture content is 9.7%, crude fibre (3.5% to 4.5%), fat (1% to 1.5%), ether extract 1.2%, carbohydrate (59% to 65%) and ash content is (4.5% to 5.5%) (Tsou *et al.*, 1979). Besides these, mungbean is rich in potassium, phosphorus and ascorbic acid (Vitamin A) but calcium, sodium and iron are present in a low quantity. The protein in mungbean seed contains high amount of lysine but less amount of cystine and methionine amino acids (Duke, 1983). As there is a low-fat content found in mungbean and other related legume crops but the lipids in mungbean contain, tocopherols and tocotrienols, both exhibiting the antioxidant effects (Gopala Krishna *et al.*, 1997; Mabaleha *et al.*, 2004; Zia-Ul-Haq *et al.*, 2008; Kamal-Eldin and Appleqvist, 1996). A range of anti-nutritional factors such as proteinase inhibitors, Tannins, phytic acid, trypsin inhibitors, oligosaccharides, hemagglutinins and saponins are present in the seeds of mungbean and other legume pulses which affect their biological and nutritional value. For the development and seed germination of mungbean, phytic acid (inositol hexaphosphate) is necessary and an important component of phosphorus (Dhaiya *et al.*, 2015). Among these anti-nutritional factors, phytic acid, saponins, tannins and protease inhibitors, they show antioxidant and anti-carcinogenic properties (Dai and Mumper, 2010).

The average amount of carbohydrates, proteins, lipids in mungbean grain is 61.0g/100g, 23.8g/100g, 1.22g/100g, and 4.57g/100g respectively (Watson, 1997; Mubarak 2005; El-Adawy *et al.*, 2003, Sathe, 1996). The fiber and ash amount in mungbean are 4.57g/100g and 3.51g/100g respectively (Tsou and Hsu, 1978; Shehata and Thannoun, 1980). The average amount of energy produced by mungbean is 344kcal/100g. Among carbohydrates, glucose is 0.3% in mungbean while the average quantity of other sugars like

sucrose, lignin, cellulose, hemicelluloses, amylose, and starch is 1.3%, 3.9%, 3.9%, 4.7%, 24%, and 47% respectively. In proteins, various amino acids are present in different amounts. The major constituents of proteins are alanine (4.1g/16g), arginine (5.8g/16g), aspartic acid (13.0g/16g), glutamic acid (18.3g/16g), glycine (3.6g/16g), histidine (3.2g/16g), isoleucine (4.3g/16g), leucine (7.6g/16g), methionine (1.2g/16g), lysine (6.5g/16g), phenylalanine (5.4g/16g), proline (4.5g/16g), serine (4.9g/16g), threonine (3.2g/16g), tryptophan (1.2g/16g) and valine (5.1g/16g) (Dzudie and Hardy, 1996; Abd El-Moniem, 1999).

Sathe, (1996) reported a fraction of fatty acids in mungbean that includes palmitic acid (14.1%), stearic acid (4.3%), oleic acid (20.8%), linoleic acid (16.3%), linolenic acid (35.7%) and behenic acid (9.3%). Among micronutrients, vitamins include thiamine (0.5mg/100g), riboflavin (0.3mg/100g), and niacin (2.2mg/100g) (Kylan and McCready, 1975; Abdullah and Baldwin, 1984; Prabhavat, 1990). According to Poehlman, 1991, composition of different minerals in mungbean comprises of calcium (113.4mg/100g), copper (1.0mg/100g), iron (5.9mg/100g), potassium (956.6mg/100g), and magnesium (162.4mg/100g). Dried mungbean seeds can be consumed in many ways like fermented or crushed into powder to make soups, curries, Dahl, alcoholic beverages, confections and porridge products and beans can also be used as fresh salad vegetable and food in many western countries (Lambrides and Godwin, 2007). A whole mungbean plant including seeds, pods, stem, roots, leaves and vegetative parts, all are good source of proteins, phosphorus, calcium and vitamins (AD) and can be harvested at full maturity. The digestible property and easily availability of mungbean by animals play an essential role to use it as supplements in animal feed and its forage usually considered having palatable taste (Singh *et al.*, 2013). So, mungbean can be grown as a dual-purpose crop to obtain forage and seed collectively (Karamany, 2006).

Mungbean is most liked legume crop among the Chinese people and it has been grown and utilized from the past 2000 years due to its well-known properties including detoxification activities, reducing heat stroke, gastrointestinal problems, refreshing mentality, skin moisture and many other summer heat related issues. In China, the seeds and several other parts of mungbean plant have been used in medicines to cure the diseases like gastritis, red dysentery, macula, hepatitis, cholera, uraemia, corneal opacity, liver complaints, paralysis, toxicosis, rheumatism. The roots can be used as narcotic drugs (Zheng, 2002). Legume crops possess the antitoxin effect that's why they have been utilized in medical and cosmetics since the earliest times (Sharma and Mishra, 2009). They also show antihypertensive and anti-diabetic properties (Lin *et al.*, 1974; Yang *et al.*, 2008). The medicinal and nutritional constitution of mungbean can be improved through germination. The consumption of mungbean and sprouts on daily basis not only good to maintain the nutrients

in human body but also decreases the absorption of toxic compounds, hypercholesterolemia, perpetuates microbial flora in intestine, reduces the risk of obesity and halts the cancer, diabetics and heart diseases (Kruawan *et al.*, 2012; Nakamura *et al.*, 2016).

Mungbean, *Vigna radiata*, is one of the paramount pulse crops that supplement the cereal based diet for poor societies specifically in Asian countries. Mungbean is widely distributed throughout South Asia, Southeast Asia, West Indies, tropical Africa, Subtropical Africa, Australia, North and South America. Among Southeast and South Asia, Pakistan, Nepal, India, Burma, Sri Lanka, Thailand, Bangladesh, Indonesia, Philippines, Malaysia, Taiwan, Bhutan, and Myanmar are countries contributing to mungbean production lead to enhancement of overall production of mungbean worldwide (Lakhanpaul *et al.*, 2000; Karthikeyan *et al.*, 2014; Singh, 1988; Chadha *et al.*, 2010). Globally, the area covered by pulse crops is approximately 73.2 million hectares followed by 61.72 million tons of productivity and the average production of these crops is 843 kg/ha worldwide (Pandiyan *et al.*, 2012). In 2014, production and yield of total pulse crops increased up to 77 million tones and 929 kilograms per hectare respectively stated by FAOSTAT. However, among all these pulse crops, mungbean is a vital pulse crop that covers an area of above six million hectares annually and the standard mungbean productivity is 384kg/ha (Karthikeyan *et al.*, 2014). Mungbean, enriched in protein, is mainly produced throughout Asia to overcome the protein-based malnutrition. It is a widely produced pulse crop across Asian countries since ancient times. About 90% of the total mungbean production comes from Asia. In Asia, India is the main producer and consumer of mungbean contributing to 65% acreage worldwide. India accounts for 54% of the total mungbean production globally (Singh, 2011).

In India, mungbean is cultivated across central, northern, and western parts of the country from March to September. It is grown in the southeastern part of the country from November to April. Mungbean is basically considered as native to India. The average mungbean production was 0.63 million tones covering an area of 1955 thousand hectares with an average yield of 322 kilograms per hectares in 1971. In 2013 mungbean production increased to 1.48 million tones leads to an increase in yield of 469 kg/ha with an average increase in the area of 3187 thousand hectares. India produces mungbean not only for its own consumption purpose but also for exporting to its trading partners. India imported about 0.54 million tons of mungbean to Australia, Tanzania, Uzbekistan, Mozambique and Myanmar (mungbean). In China, mungbean is cultivated only for its domestic utilization. Mungbean is consumed in noodles or as sprouts for Chinese people's diet. Mungbean is grown in different Chinese provinces including Jilin, Henan, Mongolia, and Anhui. In northern China, mungbean is cultivated in early June and harvested late in August. In the middle and lower valley of Yangtze River,

mungbean is grown in April to May and harvested in September. The average mungbean production is 0.6 million tons from 2014 to 2015 and the average mungbean yield is 1276 kilograms per hectares (Li *et al.*, 2016).

Other countries like Australia, Thailand, Bangladesh, and Cambodia also contribute to mungbean production. In Australia, mungbean is mainly produced in New South Wales and Queensland. Above 90% of mungbean from Australia is exported to Asian countries mainly India being the greatest buyer of this crop. In Thailand, mungbean is grown in an area of about 200 thousand hectares (Srinives and Somta, 2011). In Bangladesh, 32.7 thousand tons of mungbean was produced in an area of 38.8 thousand hectares by 2014 to 2015. In Cambodia, production and area covered by mungbean were 74.6 thousand tons and 62.7 thousand hectares respectively in 2012. In the United States, mungbean is known to be a good source of bean sprouts. Almost 10 million kilograms of mungbean is being utilized by the US. About 90% of the mungbean is grown in California, Oklahoma, and Texas. Area covered by mungbean production is approximately 50,000 hectares (Bhardawaj *et al* 1999).

In Pakistan, majorly produced summer and winter pulse crops are mungbean, black gram, red gram and chickpea, lentils, field pea respectively (Habib *et al.*, 2013). It is grown in the spring season (March-June) and a rainy season (July-October). Mungbean is a foremost, short duration, warm seasonal legume crop being used as a supplement of cereal-based crops in Pakistan. It is a widely grown crop all over Pakistan especially in Punjab and other provinces of Pakistan that include Sindh, Baluchistan, and Khyber Pakhtunkhwa (Haqqani *et al.*, 2000). In Punjab, mungbean is cultivated in different districts including Bhakkar, Layyah, Narowal, T.T Singh, Kasoor, Nankana Sahib, Sheikhupura, Chakwal, Jehlam, Sialkot, Mianwamman, Faisalabad, Muzaffargarh, Dera Ghazi Khan, and Dera Ismail Khan. In the Federal region, mungbean is grown in Islamabad, Rawalpindi, and Attock. In Sindh, it is cultivated in Sajawal, Thatta, and Larkana (Iqbal and Mukhtar, 2014).

In Pakistan, 20.40 million hectare area is under cultivation out of the total geographical area of about 79.61 million hectares. The area under productivity has increased from 100,000 hectares to above 200,000 hectares hence increased in production from 50,000 metric tons to 100,000 metric tons since 2000 (Weinberger, 2005). In 2002, about 104500 tons of mungbean was produced by an area of 219200 hectares (Rashid *et al.*, 2004). Government of Pakistan, 2003 gave data about an area of 239,200 ha under cultivation of mungbean which produces 115,400 tons of mungbean with an average yield of 482 kg/ha. In 2015, mungbean is cultivated over an area of 127,500 hectares. The total area under production in Pakistan is 135,000 hectares and 90,000 tons of mungbean give a yield of 662.25kg/ha. Punjab alone accounts for 85% of the area for mungbean production. About 87% of total mungbean production comes from Punjab. In Punjab, 116,000

hectares area is under production. In 2012-13, about 78,000 tons of mungbean is produced followed by a yield of 672.09 kg/ha. In Sindh, 0.90 tons of mungbean is produced over an area of 2,000 hectares with an annual yield of 428.57 kg/ha. In KPK, the area under cultivation, production, and yield of mungbean is 7,000 hectares, 4,000.40 tons, and 619.72 kg/ha respectively. In Baluchistan, mungbean is grown over an area of 10,000 hectares. The total annual production and yield of mungbean are 6000.20 tons and 626.26 kg/ha respectively. In NWFP-North West Frontier Province, mungbean is grown over an area of 9500 hectares to produce grains of 6000 tons (Government of Pakistan, 2015).

Stresses of mungbean crop: Although the major constraints of leguminous crops are the biotic stresses including fungi, bacteria, insect pests, nematodes, and viruses or abiotic stresses including salinity, freezing, waterlogging, and drought. These factors can drastically damage the productivity of legume crops. The crops that are already under abiotic stress are highly vulnerable to pathogen attacks (Reddy *et al.*, 2004). The yield loss can be occurred up to 10 to 100% due to the viruses and fungal attacks. Coyne *et al.*, (2003) described the viruses which are usually found in common beans are BGMV (Bean Golden Mosaic Virus), BCMNV (Bean Common Mosaic Necrotic Virus), and BCMV (Bean Common Mosaic Virus). Similarly, legume plants are more susceptible to pathogens when there is waterlogging stress. The uptake of several minerals (such as sodium, manganese, iron and potassium) can be reduced due to waterlogging (McDonald and Dean, 1996). To overcome these stresses, several chemicals including fungicides and insecticides have been used but due to environmental hazards, these approaches are avoided. So, the development of resistant cultivars is an effective approach to deal with these stresses. Several biotechnological techniques are preferred over conventional breeding for a better selection of resistant varieties. These techniques include genetic transformation, tissue culture, molecular marker-assisted selection (MAS) and in vitro mutagenesis (Dita *et al.*, 2006).

Mungbean crop is susceptible to many biotic and abiotic factors hence decreasing the overall mungbean production and causing great economic losses. Mungbean must tolerate this stressful environment to survive. Unable to survive this stressful environment leads to disturbance in the functionality of mungbean systems. These stresses affect cellular homeostasis, disrupt metabolism, and interrupt the biochemical and physiological processes of mungbean. Abiotic factors include drought stress, waterlogging stress, temperature stress, salinity stress, and many other stresses (Arora *et al.*, 2002; Srivalli *et al.*, 2003). In drought stress, the physiological state linked with development, growth, and economic yield of mungbean is affected. Water loss basically affects the turgor pressure of the mungbean plant hence loss in turgidity cause a decrease in cell growth. Mungbean is more susceptible to drought stress than other pulses because

it requires more water for its cultivation. So, its productivity is more affected in drought stress season like summer and spring season (Singh and Singh, 2011).

Waterlogging stress mainly disrupts the early stages of development in the mungbean plant. Mungbean plays an important role in atmospheric nitrogen fixation to fulfill its own nitrogen demand and many other surrounding plants. Waterlogging or flooding reduces the ability of nodule activity and nitrogen fixation. Winds coupled with heavy rainfall caused extensive yield loss by damaging mature mungbean crops (Ali and Kumar, 2006). Mungbean production is greatly affected by a change in temperature and photoperiod. Elevated temperature stress has a negative impact on flower retention and pod formation. High temperature leads to an increase in flower shedding and a decrease in seed production due to a lack of reproduction and pollen grain's inviability (Kumari and Verma, 1983; Rainey and Griffiths, 2005).

Salinity stress cause a decline in shoot and root length, no branches and root hairs, seed germination, and seedling that leads to a decrease in mungbean production (Saha *et al.*, 2010). Salt stress causes distinct symptoms like a decrease in the content of pigment chlorophyll A, B, and carotenoids and enhanced necrosis and chlorosis (Wahid *et al.*, 2007). Other abiotic stresses include ultraviolet and ionizing radiations, metal pollutants, insecticide residue, prolonged rainy conditions, and many other mechanical factors like wind, water pressure (HanumanthaRao *et al.*, 2016). These abiotic stresses make mungbean crops more vulnerable to biotic stresses.

Biotic stress plays an important role in agriculture as it immensely damages agricultural products that lead to malnutrition in different regions of the world (Moustafa-Fraag *et al.*, 2020). Biotic stress includes different organisms like bacteria, fungi, viruses, pests, nematodes, weeds, and parasites. These organisms are of great economic importance because of their impact on mungbean plants especially in Asia (Taylor *et al.*, 1996; Singh *et al.*, 2000; Raguchander *et al.*, 2005; Mbeyagala *et al.*, 2017; Pandey *et al.*, 2018). Major viral diseases of mungbean are mungbean yellow mosaic virus (MYMV), cucumber mosaic virus (CMV), alfalfa mosaic virus (AMV), mosaic mottle virus (BCMV), mungbean leaf curl virus (MLCV) and leaf crinkle virus (ULCV) (Singh *et al.*, 2018).

The most important fungal diseases of mungbean are powdery mildew caused by *Podosphaera fusca* and *C. truncatum*, Cercospora leaf spot (CLS) caused by *Cercospora canescens* and anthracnose disease caused by *Collectotrichum acutatum*, *C. gloeosporioides*, and *C. truncatum*. Dry root rot in mungbean is now considered as the emerging disease caused by *Macrophomina phaseolina*. Other fungal diseases are Fusarium wilt, web blight, and Alternaria leaf spot caused by *Fusarium solani*, *Rhizoctonia solani*, and *Alternaria alternate*, respectively (Ryley and

Tatnell, 2011; Pandey *et al.*, 2018). The main bacterial diseases of mungbean are bacterial leaf spot, halo blight and tan spot caused by *Xanthomonas campestris*, *Pseudomonas syringae*, and *Curtobacterium flaccumfaciens*, respectively (Pratap *et al.*, 2020).

Insect-pests may directly affect the mungbean plant for feeding purposes or indirectly for being a vector for many pathogens. Insect-pests adversely damage mungbean production by attacking the crop from sowing to cultivation period and till the end step of storage. For mungbean, most important insect-pests are thrips, whitefly, aphids, stem fly, pod bugs, bruchids, and pod borer complex (Swaminathan *et al.*, 2012). Stem fly is one of the chief insect-pest that takes an adverse toll on mungbean yield. Mungbean is mainly infested by *Ophiomyia phaseoli* specie of stem fly. Other common species are *Ophiomyia centrosematis* and *Melanagromyza sojae* (Talekar, 1990). Thrips are also economically important insect-pests that damage mungbean crops. They affect mungbean in both seedling and flowering stages. The thrips *tabaci* and thrips *palmi* are pests of the seedling stage while *Caliothrips indicus* is the pest of the flowering stage. The *Maruca vitrata* specie of spotted pod borer present in the tropical and subtropical regions causes immense damages to mungbean (Zahid *et al.*, 2008).

These diseases cause severe losses in mungbean production and ultimately in its yield. MYMVD is the most destructive disease of mungbean taking up to 100% yield loss. In India, the decline in mungbean production due to MYMVD is approximately 85% (Karthikeyan *et al.*, 2014). In Pakistan and India, about a 10-44% decrease in mungbean yield is caused by Dry root rot disease (Kaushik and Chand, 1987; Bashir and Malik, 1988). Almost 30-70% of mungbean yield loss as a result of anthracnose (Kulkarni, 2009; Shukla *et al.*, 2014) and 33-44% as a result of Rhizoctonia (Singh *et al.*, 2013) is reported in India. CLS disease causes about 97% yields to loss in India and Pakistan (Iqbal *et al.*, 1995; Chand *et al.*, 2012; Bhat *et al.*, 2014). A fungal disease, powdery mildew, reported causing about 40% decrease in yield (Khajudpam *et al.*, 2007). Other trivial fungal diseases include Fusarium wilt and Alternaria leaf spot that take a high toll on mungbean yield and cause 20% (Anderson, 1985) and 10% loss respectively (Maheshwari and Karishna, 2013).

A bacterial disease, halo blight, is considered a rising disease in Australia (Noble *et al.*, 2019) and China that causes a 30-50% reduction in mungbean yield specifically in China (Sun *et al.*, 2017). The prevalence of bacterial leaf spot, caused by *X. phaseoli*, is reported to be 30% in India (Kumar, 2016) and 70% in Iran (Osdaghi, 2014). The pests accounted for a 2-84% yield loss worth of 30 million dollars (Zahid *et al.*, 2008). Among pests, bruchids can damage the entire mungbean population leading to the crop loss up to 100% (Tomooka *et al.*, 1992; Somta *et al.*, 2007).

About 47% of emerging infectious diseases have been caused by viruses in plants (Yadava *et al.*, 2010). Among all the

infectious diseases, YMV is the group of pathogens that severely affect the mungbean crop production. YMV belongs to the genus *begomovirus* from the *Geminiviridae* family of viruses. In Pakistan, yellow mosaic virus disease was first reported in the territory of Lyllpur also known as Faisalabad (Vasudeva, 1942). YMVD was first reported in the lime bean from western India and then in mungbean from northern India (Nariani, 1960). Yellow mosaic virus disease in mungbean was first named by Nene (1968) as a mungbean yellow mosaic virus disease. Later on, MYMVD was also confirmed in Pakistan (Ahmad, 1975), Bangladesh (Jalaluddin and Shaikh, 1981) Thailand, (Thongmeearkom *et al.*, 1981) and many other countries of Asia like Iran, Nepal, Malaysia, Guinea, Vietnam, and Indonesia. A high incidence of MYMVD in green gram was reported by Murugesan and Chelliah (1977) during the summer season.

There are important species of *begomoviruses* that are responsible for causing MYMD in mungbean crops namely as Horsegram yellow mosaic virus, Dolichos yellow mosaic virus, mungbean yellow mosaic India virus, and mungbean yellow mosaic virus. These plant-infecting *begomoviruses* have geminate particles of 30nm long in length and 18-20 nm in width. These viruses with circular and single-stranded genome have an icosahedral structure containing 110 indistinguishable protein subunits and 22 capsomeres. The size of the *begomoviruses* genome is approximately 2800 nucleotides (Qazi *et al.*, 2007; Malathi and Jhon, 2008; Ilyas *et al.*, 2010). They mainly affect dicotyledonous crop plants like soybean, black gram, moth bean, urdbean, yard-long bean, horsegram, and common bean (Haq *et al.*, 2011).

Mungbean yellow mosaic viruses can be monopartite having single DNA or bipartite having two DNA components named as DNA-D & DNA-B. The size of components A and B is approximately 2.7kb and 2.6kb respectively. DNA-A component performs its role specifically in the nucleus. This component encodes for the genes that are responsible for the replication process, transcription, regulation of transcription, viral genome encapsidation, and transmission of different insects. The important proteins associated with replication are rep protein for initiation of replication by rolling-circle mechanism, DNA helicase for pulling apart double-stranded DNA by breaking hydrogen bonds and REN protein involves in replication enhancement. TrAPs-transcriptional activator proteins and CPs-coat proteins are required for gene regulation and encapsidation, respectively (Choudhry *et al.*, 2006; Qazi *et al.*, 2007). V2 protein is reported to play an important role in movement and C5 protein blocks the defence mechanism of a plant by PTGSp post-transcriptional gene silencing. The other component, DNA-B, encodes for two genes nuclear shuttle protein and movement protein. These two proteins, NSP and MP, play an exclusive role in viral movement between the cells of a single plant (Shivaprasad *et al.*, 2005).

Whiteflies serve as a vector for many bacterial and fungal diseases, but recent studies show that whiteflies are also serving a potential vector for transmission of *begomoviruses* in beans. The disease intensity is associated with the whitefly population. They are mainly present in tropical, subtropical countries and temperate agricultural areas (Costa *et al.*, 1976). Control of whiteflies is one of the substantial challenges to control YMVD. This disease can also be controlled by using systemic insecticides, cultural and biological practices (Verma *et al.*, 1992).

Bemisia tabaci is considered to be native to Pakistan and India reported by Brown *et al.*, 1995. Almost 600 plant species act as a host for *Bemisia tabaci*. The prime vector for MYMV is *Bemisia tabaci*, a polyphagous and cosmopolitan pest. MYMV is not transmitted directly through soil and seed. *Bemisia tabaci*, whitefly, causes direct damage to mungbean through feeding as it is phloem-feeding pest or indirect damage by transferring plant pathogens (Oliveria *et al.*, 2001). Whitefly is a tremendously persistent and efficient vector for transmission of MYMV because of its latent period of about four days. A viruliferous adult can transmit the dreadful MYMV within an attainment and inoculation access duration of about 24 hours. In some cases, acquisition and inoculation because of whitefly adults can be done in less than 15 minutes. The whitefly has the capability to attain the MYMV even after a single bite that increases its efficiency of transmission on other mungbean crop plants (Malathi and John, 2008/9). The most competent male and female whitefly adults in insect population can have the capability of infection for three and ten days respectively. So, it proves female adults are far more efficient than male adults. Although both female and male adults can't have infectivity capacity throughout their entire life. The nymph stage of the whitefly can get the MYMV from the infected leaves but cannot it to the eggs of whitefly. The population of whitefly is considered to be high during the summer season as compared to the rainy and spring season. High temperature attributes the favorable conditions for the whitefly multiplication within the host plant (Karthikeyan *et al.*, 2014).

The particles of MYMV aggregate in nuclei of phloem leaf cells of mungbean and then multiply. These viral aggregates sometimes cover the total volume of infected phloem cells. Viral particles can be in a scattered form or aggregated form containing cylindrical or paracrystalline arrangement in vacuoles of sieve elements (Thongmeearkom *et al.*, 1981). Initial symptoms associated with MYMVD are small pale-yellow specks in a yellow mosaic pattern around the vein and then these specks spread in the entire leaf. Severe infestation leads to chlorosis of the entire leaf. An infected leaf is characterized by asymmetrical green and yellow patches. The infected leaves become dry, wither, and shriveled. Pods of infected mungbean are small in size bearing small, immature, deformed, and desiccated seeds decreasing the photosynthesis rate and consequently the yield both quantitatively and

qualitatively (Malathi and John, 2008). The magnitude of yield loss depends on the severity and intensity of the disease on the individual mungbean plant, number, and stage of a plant at which it gets affected (Min *et al.*, 2020). Approximately 10% to 85% yield is reduced due to MYMVD (Grewal JS, 1988; Verma *et al.*, 1992; Khattak *et al.*, 2000; Kang *et al.*, 2005).

Management strategies for abiotic and biotic stresses: Moth bean, soybean, mungbean, common bean, pigeon pea and cowpea, these are all important hosts of MYMV. The control of MYMVD is an important strategy to overcome the crisis of mungbean production. There are several control strategies to manage the YMVD: controlling the vector for MYMVD, providing the alternative host plants or weeds for a virus, altering cultural practices that are unfavorable for the MYMVD occurrence, and developing the resistant or tolerant varieties of mungbean plants. The disease can be greatly reduced by controlling the whitefly population (Varma *et al.*, 1992; Karthikeyan *et al.*, 2014; Min *et al.*, 2020). However, The disease severity can be reduced to some extent through various management strategies including cultural practices and by using chemicals (pesticides and insecticides). So, it is quite difficult to eliminate this disease completely and through the continuous use of chemicals there may be a harmful impact on the environment (Iqbal *et al.*, 2011). The most commonly used chemicals such as Tracer, Mycotal and Imidacloprid were considered effective against MYMV and its vector. Among all these three chemicals, Imidacloprid chemical was most effective against MYMV and whitefly (Khan *et al.*, 2012). During severe infection, these chemicals are unable to control the disease.

Reducing the population of whitefly is a valuable strategy to control the MYMVD. The management of whitefly is very difficult because of its attacking ability in hoarding rather than individual ones. Therefore, hordes of thousands of whiteflies can severely attack mungbean plants and weaken them. The vector (whitefly) can be controlled by various management strategies such as chemical, mechanical, botanical, and cultural. Among all these methods, control by chemicals is the prime method in managing the vector population. In chemical control, the most commonly used systemic insecticides are triozophos, acetamiprid, imidacloprid, and ethion. These chemicals can either kill whiteflies on contact or can be taken inside the mungbean plant to protect it from further attack in upcoming days at least for a few weeks (Wang *et al.*, 2009). Younger stages of whiteflies are more responsive to insecticides comparing with the adult stages. A specific level of a certain insecticide can be toxic enough against the nymph stages of the whitefly because nymph stages are more susceptible. Incomplete exposure leads to the failure of sustainable control so the application of chemicals must be well defined for complete exposure such as imidacloprid is applied on the underneath of the leaves where nymph stages are present (Ghosh, 2008). Cultural practices in the

management of whitefly are useful for only with its low population. Removal of weeds and crop residues is a helpful strategy to keep the field sanitation against whitefly. The population-level can also be controlled by the removal of leaves of infested plants with hands and by using syringing. Intercropping in mungbean with other crops such as wheat, sunflower, cotton, maize, and sugarcane can also be useful for the management of MYMVD (Qazi *et al.*, 2007; Karthikeyan *et al.*, 2014).

The development of resistant varieties of mungbean is also a promising way to combat MYMVD. Resistant varieties can be produced by biotechnological applications such as breeding through genetic engineering, genetic transformation, marker-assisted selection, RNA-interference technology, gene editing and pathogen-derived resistance method. In mungbean, resistance against MYMVD is directed by a single dominant gene (Sandhu *et al.*, 1985), single recessive gene (Reddy and Singh, 1995; Saleem *et al.*, 1998; Reddy, 2009), complementary recessive and two recessive genes (Pal *et al.*, 1991; Ammavasai *et al.*, 2004). Pathogen-derived resistance is also an effective method for controlling MYMVD (Sanford and Jhonston, 1985). In this method, viral genes for coat protein, protease, replicase, nuclear shuttle protein, and movement protein are expressed in the mungbean plant and then these genes protect plants against the MYMV (Shivaparasad *et al.*, 2006). Haq *et al.*, (2010) reported that a gene called Rep (initiation protein for replication) which is responsible for virus replication was targeted and silenced. Hence, resistance against MYMIV was developed.

Marker-Assisted breeding of mungbean: Marker-assisted selection is considered the most promising technique for the selection of resistant varieties rather than field-based selection through breeding. Marker-based selection of the resistant varieties of mungbean is a more reliable, consistent, and time-saving technique. Molecular markers assisting in breeding research are restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), Inter-simple sequence repeats (ISSR), resistance gene analog (RGA), sequence characterized amplified region (SCAR), single nucleotide polymorphism (SNP), short tandem repeat (STR), expressed sequence tag (EST), variable number tandem repeat (VNTR) and sequence-tagged sites (STS). These markers are neutral, co-dominant, economical, comprehensible, quick, highly informative, automated and in large number present in nuclear, mitochondrial and chloroplast genome. The construction of QTL maps and genes associated with disease mainly MYMV resistance have been studied through DNA molecular markers (Selvi *et al.*, 2006; Chen *et al.*, 2013; Dhole and Reddy, 2013; Gupta *et al.*, 2015).

The use of genetic markers linked with MYMVD resistant genes is a successful approach as compared to conventional breeding for the selection of mungbean resistant varieties. In

conventional breeding, resistant varieties are selected based on their phenotypic traits making this approach more time consuming, less effective and most importantly it demands evaluation based on hot-spot regions. Molecular markers based indirect selection is cost-effective, time-saving, accurate, and requires no screening of resistant varieties linked to MYMVD (Selvi *et al.*, 2006; Karthikeyan *et al.*, 2012). Resistant genotypes can be detected even in the seedling stage of the mungbean plant itself without developing the resistant varieties that is time taking process (Selvi *et al.*, 2006).

Breeding against abiotic stresses is quite difficult due to the presence of complex traits. In common bean, marker-assisted selection (MAS) is useful against the drought stress (Schneider *et al.*, 1997). According to Ouedraogo *et al.*, (2002) an amplified fragment length polymorphism (AFLP) named as E-AGA/M-CTA₄₆₀ is linked with parasitic weed resistance gene Rsg3 in cowpea. Similarly, another molecular marker, SCAR (SEACTMCAC83/85 has been reported in cowpea legume crop. This marker is also associated with parasitic weed resistance Rsg1 gene (Boukar *et al.*, 2004).

Molecular markers linked to resistant genes have been identified for various viral diseases (Urrea *et al.*, 1996; Marczewski *et al.*, 2001; Zheng *et al.*, 2003; Gao *et al.*, 2004). Many molecular markers linked to MYMVD like RFLP, SSR (Kumar *et al.*, 2002), RAPD, ISSR (Kalaria *et al.*, 2014), SCAR (Dhole and Reddy, 2013) and RGA (Basak *et al.*, 2004) are developed for mungbean. In some cases, the RAPD marker was either used directly in mungbean germplasm for the detection of MYMV-linked resistant genes or indirectly by converting it into a SCAR marker (Selvi *et al.*, 2006; Chen *et al.*, 2007). The outcomes of screening of mungbean germplasm may be in the development of resistant, tolerant, or susceptible varieties. SCAR marker was also identified in many other crops such as common beans (Alzate-Marin *et al.*, 1999), soybean (Zheng *et al.*, 2003) and blackgram (Souframanien and Gopalakrishna, 2006).

RAPD markers have been used to identify resistance genes linked to MYMV in French bean (Ravishankar *et al.*, 2009) and in mungbean crop (Selvi *et al.*, 2006). Bulk segregant analysis (BSA) linked to RAPD marker, was used to examine resistant and susceptible individuals of F_{2:3} populations. Almost 35 mungbean genotypes were screened out against MYMV. OPP 07, a RAPD primer, was indicated a definite band of about 895bp in the resistant parent genotype but this band was absent in susceptible parent genotype. Furthermore, to check that OPP 07₈₉₅ RAPD marker was linked with MYMV resistance, co-segregation analysis was carried out in susceptible and resistant genotypes of mungbean. This molecular marker can also be helpful to identify Quantitative trait loci (QTL) associated with MYMV resistance (Dharajiya and Ravindrababu, 2019).

Chen *et al.*, (2013) described that three major quantitative trait loci (QTLs) linked to MYMV resistance genes and one major

QTL against bruchid resistance have been identified. A linkage map was developed by using various DNA molecular markers (SSR, RAPD, AFLP and SCAR markers). Precise genetic linkage maps are required for the accurate mapping of quantitative trait loci (QTLs) associated with the MYMV resistance. In this study, GBS technology was used for the development of genetic linkage map. The resulting genetic linkage map showed 538 single nucleotide polymorphism (SNP) markers. These markers were randomly distributed throughout the genetic linkage map and each chromosome in map can have 30 to 79 SNP markers. After the studies, QTL analysis by using the genetic linkage map indicated a MYMV resistance linked QTL on chromosome 4, named as qMYMV 4-1. This QTL region in the genome is responsible for the controlling of MYMV resistance (Mathivathana *et al.*, 2019). Resistance genes linked to disease show a high level of polymorphism, thus these highly polymorphic regions are identified by using specific primers for amplification in PCR (polymerase chain reaction) (Michelmore *et al.*, 1991). These highly polymorphic regions serve as a molecular marker and once these molecular markers are recognized they can be used to generate the MYMV resistance linked QTLs in the mungbean germplasm (Yu *et al.*, 1996). Molecular markers are used for tagging resistance genes linked to a specific MYMVD in molecular-based plant breeding (Selvi *et al.*, 2006; Karthikeyan *et al.*, 2012). RAPD marker is extensively used for detecting and exploiting genetic diversity as it is a dominant marker, inexpensive, simple, fast, and reliable in use (Williams *et al.*, 1990; Harris, 1999; Selvi *et al.*, 2006). Bulk segregant analysis (BSA), a marker-linked technique, is used to detect the molecular markers associated with the genes conferring resistance or susceptibility against a disease. This analysis was also used to identify the RAPD markers linked to resistant genes of various diseases like angular leaf spot disease in common beans (Nietsche *et al.*, 2000), leaf rust disease in wheat (Tar *et al.*, 2002), downy mildew disease of soybean (Chowdhury *et al.*, 2002) and rust disease in common beans (Park *et al.*, 2004) and mungbean yellow mosaic virus disease in mungbean (Selvi *et al.*, 2006; Karthikeyan *et al.*, 2012).

BSA was carried out to analyze the individuals of the F₂ generation of resistant KMG 189 × susceptible VBN (Gg) 2 for the screening and identification of the molecular marker in mungbean linked to the MYMV resistant gene. Results showed that among 203 individuals of F₂ generation 30 individuals were resistant, 41 moderately resistant, 56 MYMV susceptible, 57 moderately susceptible individuals, and 19 highly MYMV susceptible individuals. Further 72 decamer oligonucleotide primers of the random sequence were used for the analysis of parental polymorphism. From 72 primers, 55 primers showed the DNA amplification but only 11 primers showed results of parental polymorphism. Among these 11 primers, OPBB 05 (5'-GGGCCGAACA-3') primer polymorphism OPBB 05 260 (band of 260bp) in

resistant and susceptible bulks. This marker was further confirmed by co-segregation analysis. The results showed that OPBB 05₂₆₀ RAPD marker is strongly linked to MYMV in mungbean and can also be converted into a SCAR marker for identification and selection of resistant genotypes (Karthikeyan *et al.*, 2012).

A study was carried out to identify the MYMVD linked RAPD marker in French bean. Bulk segregant analysis was done for the identification of RAPD marker associated with resistance against MYMVD. In this analysis, 140 primers (OPP 01-20, OPG 01-20, OPS 01-20, OPR 01-20, OPY 01-20, OPW 01-20, OPX 01-20) were used for identifying polymorphic regions in the DNA bulks of susceptible F2 and resistant individuals and their parents. These primers showed 98% of the DNA amplification between resistant and susceptible bulks and their parents. Specific bands were produced by 22 primers for the resistant individuals but not for the susceptible one. Among 22 primers, only four primers OPP 7, OPG 13, OPX 5 and OPW 17 generated particular disease resistant bands OPP 7₇₃₀, OPG 13₄₅₈, OPX 5₆₇₀ and OPW 17₃₈₀ respectively in resistant bulk and resistant parent but not in susceptible bulk and susceptible parent.

The study was conducted to detect molecular markers linked to resistance genes against MYMVD by using ISSR technique. Previously, the ISSR technique has also been used in many crops such as beans, maize, rice, sorghum, wheat, and barley for tagging resistance genes linked to biotic and abiotic factors (Reddy *et al.*, 2002). ISSRs are preferable over other markers because of its high capacity to reveal genetic polymorphism and offer great potential to find out inter- and intra-genomic diversity (Zietkiewicz *et al.*, 1994). These markers are identified by using specific primers. In general, primers with GA, AG, TC, CT, CA and AC repeat show comparatively high polymorphism than primers with sequence of di, tri or tetra nucleotide repeats. The dinucleotides of AT repeats are more frequent in plants but the AT repeats-based primers would self-anneal rather than anneal with the DNA template and as a result would not amplify. Tri and tetra nucleotides are less abundant and use of these repeats in ISSR markers is comparatively low than the dinucleotide repeats (Reddy *et al.*, 2002). Studies on resistance genes linked to a specific disease have showed a high level of SSRs presence and polymorphism at specific loci (Yu *et al.*, 1996). Therefore, ISSRs based on polymorphism and SSRs could provide resistance genes-linked molecular markers against diseases. In this study, the ISSR approach was used for detecting markers linked to the resistance genes against MYMV in blackgram. Hari *et al.*, (2017) reported that genetic diversity analysis was done among 17 resistant and susceptible genotypes of mungbean through SSR or microsatellite markers. The results showed very low genetic variation of about 58.8% polymorphism by using 29 SSR primers.

Marker-based resistance genes have been tagged in many other crops like Phaseolus (Urrea *et al.*, 1996), pea (Gao *et al.*, 2004) and soybean (Jeong *et al.*, 2002; Zheng *et al.*, 2003; Jeong and SaghaiMaroof, 2004). The ISSR markers can be transformed into simple SCAR markers for significant application in MAS breeding. This can be done by characterizing the disease-linked markers and designing the primers specific to its locus (Paran and Michelmore, 1993). This conversion has been effectively applied in several crops such as soybean (Zheng *et al.*, 2003) and common bean (Adam-Blondon *et al.*, 1994; Park *et al.*, 2004). The detection of a disease-linked marker and an appropriate way for large populations screening is the necessary requirement for plant breeding based on marker-assisted selection (Gupta *et al.*, 1999).

In previous studies it has been reported that, black gram var. silvestris was crossed with black gram (cv. TU 94-2) to generate mapping population (F8) of a recombinant inbred line (RIL) against MYMVD. The ISSR marker approach was employed for identifying molecular markers linked to resistance genes for MYMV. About 54 primers out of 100 primers showed amplification but only 36 from these 54 primers indicated polymorphism between black gram var. silvestris which is susceptible and the parent TU 94-2 which is resistant. Each plant from 53 RIL (F8) populations was analyzed and an ISSR marker (ISSR811₁₃₅₇) at 6.8 cM was detected linked to the MYMVD. BSA showed that the ISSR811₁₃₅₇ marker and both the phenotype segregated in a ratio of 1:1. After the sequencing of ISSR811₁₃₅₇, forward (YMV1-F) and reverse (YMV1-R) SCAR primers were designed for amplifying this marker. Screening of SCAR marker in RIL population predominantly distinguishes the resistant and susceptible varieties against MYMVD. The ISSR811₁₃₅₇ marker will be helpful for developing the MYMV-linked resistant genotypes in black gram. The ISSR811₁₃₅₇ converted into SCAR marker will be used for developing cleaved amplified polymorphism markers (CAPS). This marker linked to MYMVD will also be useful for studying yellow mosaic disease in mungbean because it is closely related to black gram (Souframanien and Gopalakrishna, 2006). Various studies were carried out for studying the inheritance of resistance gene and identification of the molecular markers linked with MYMVD by using F1, F2 and 167 F2:8 recombinant inbred lines (RILs). This RIL population was generated by crossing between 9 Mulmarada which is susceptible and 'TM-99-37' which is resistant variety. The F1 generation was susceptible, the segregation of F2 in 3S:1R phenotypic ratio and the segregation of RILs in 1S:1R ratio in the field screening indicated that the resistance gene linked with MYMV is directed by a single recessive gene. Results indicated that the 45 RAPD primers out of 140 primers showed genetic polymorphism in parental varieties and these varieties were screened by using BSA technique. Out of 45 primers, only three RAPD primers amplified

particular polymorphic regions which were OPB-07600, OPB-12820 and OPC-061750. The marker OPB-07600 at locus of 6.8cM was strongly associated with the resistance genes against MYMVD rather than the OPB-12820 marker at the locus of 25.2cM and OPC-061750 marker at locus of 22.0cM. This specific resistance-linked opb-07600 marker was cloned, and then sequenced. RAPD markers are not much stable and reproducible so these were converted into reproducible, specific, and stable sequence characterized amplified region (SCAR) markers. This SCAR marker (MYMVR-583) could increase efficiency and accuracy for selecting YMD-resistant varieties in segregating population (Dhole and Reddy, 2012).

For MAS approach, after the identification of the marker its validation was studied in 25 genotypes with various genetic backgrounds. Molecular marker validation is the practice of investigating and exploring the behavior of molecular markers and related polymorphism in different genetic backgrounds (Gupta *et al.*, 1999). In this study, the validation of the SCAR marker MYMVR-583 associated with the resistance genes against MYMV in different genotypes of mungbean showed reliable association of this SCAR marker in the resistant genotypes and absent in the remaining ten susceptible genotypes against MYMVD. These results confirmed the marker association with the resistance gene linked to MYMV in different genetic backgrounds. The SCAR marker (MYMVR-583) associated with a MYMV-linked recessive gene could hasten the development of resistant, high-yielding varieties through MAS approach (Dhole and Reddy, 2012). In other studies, single recessive gene linked to resistance against MYMV has been described in black gram (Kundagrami *et al.*, 2009) and mungbean (Reddy and Singh, 1995; Singh and Patel, 1977; Thakur *et al.*, 1977; Basak *et al.*, 2004).

Different studies had been conducted to identify the molecular markers linked to MYMVD resistance in different mungbean varieties. The identification molecular marker is necessary for studying the inheritance pattern of linked resistance genes for MYMV. After that the validation of resistance locus inheritance in the mapping population was done by the identified SCAR marker. Inclusively, 413 germplasm entries were gone through natural field infection in Vamban (the hot-spot area of MYMV) and 13 preferred resistant lines were gone through infection by *Agrobacterium* using strains retaining partial genome of VA221 and VA239 (the two different MYMV isolates). Studies showed that the two lines, VBN(Gg)2 and KMG189, had contrasting traits morphologically. VBN (Gg)2 was the recipient susceptible variety and KMG189 was the donor resistant variety. Among the 13 resistant lines, KMG189 indicated resistance to VA221 based on strain specificity. The cross between VBN (Gg)2 and KMG189 has developed ninety genotypes of F2 generation. The segregation population based on Mendelian ratio showed 3S:1R. The genetic and molecular analysis was

conducted using SCAR marker and showed that the resistance in KMG189 is due to single recessive gene (Sai *et al.*, 2017). After screening, results showed that among the two markers, UBC815707 bp and OPBE9306 bp, the later was strongly associated with resistance genes for MYMV. The former, UBC815707, was 5.56 cM away from resistance gene linked to MYMVD. Identification of molecular markers at the vicinity of 5–10 cM from the resistant gene (gene of interest) could provide resistance with great accuracy (Hittalmani *et al.*, 1995). So, the amplified products of these destined markers were converted into SCAR markers, CM815 and CM9 for more reliability and reproducibility. The SCAR marker, CM9 showed zero recombination making it strongly associated with MYMV resistance while the other SCAR marker, CM815 found at 5.56cM also linked to MYMV resistance. The CM9 marker was found on chromosome 3 in mungbean. This novel locus provided great opportunities for developing resistance against viruses in *Vigna* species (Sai *et al.*, 2017). Soybean mosaic virus disease is economically significant because of its impact on soybean production worldwide especially in China. A number of genetic markers linked to resistance genes for SMV have been recognized in soybean and many other crops. Several loci Rsv1, Rsv3 and Rsv4 linked to SMV disease have been identified in different soybean lines. Two RFLP molecular markers that are pK644a and pA186, strongly associated with Rsv1 at a map distance of 2.1 and 1.5cM. One SSR molecular marker found to be linked with Rsv1 at distance of 0.5cM on MLG F line of soybean (Yu *et al.*, 1994). The A519 RFLP marker at the locus of 0.9cM and Mng247 at the locus of 0.8cM were found to be linked with Rsv3 in MLG B2 line of soybean (Jeong *et al.*, 2002). Other SSR markers, Satt 558 at locus of 4.8cM and Satt 542 at locus of 4.7cM were linked to Rsv4 in soybean (Hayes *et al.*, 2000). These molecular markers will provide opportunities for MAS approach in breeding for producing resistant varieties and also the mapping of resistance-linked genes.

The OPN11980 and OPN1070 were two RAPD markers that have co-dominant characteristic. This marker was generated through bulk segregant analysis of the F2 generation that was obtained by crossing ICGR95-5383 × HB1. Studies showed that this marker is highly associated with the resistance genes against SMV disease in ICGR95-5383. This RAPD marker (OPN11980) with size of 980 bp was found in the resistant bulk, resistant parent line ICGR95-5383 and resistant plants of F2 generation. The other marker, OPN11070, with a size of 1070 bp was found in necrotic plants of F2 generation by crossing between resistant and susceptible varieties and plants of F1 generation. BSA of the F2 population for the RAPD molecular marker discovered that OPN11980 and OPN11070 are closely related to SMV-linked resistance genes AT a 3.3 cM map distance. After that these RAPD markers were cloned and then sequenced for converting them SCAR markers. For that conversion, well-defined primers were designed that

converted these two RAPD markers into SCN11₉₈₀ and SCN11₁₀₇₀. SCAR analysis has confirmed the association of these markers to resistance genes against SMV at the same loci. The OPN11₉₈₀ could also be used as a RFLP probe for hybridization of DNA in soybean. Southern hybridization analysis indicated that genome of soybeans contains very low number of these OPN11₉₈₀ sequences (Zheng *et al.*, 2003).

A research was conducted for identifying the genetic markers (RAPD and SCAR) linked to MYMV diseases in inter-specific crossing between VRM (Gg) 1 (a mungbean variety) X TNAU RED (a ricebean variety). The survey was conducted to identify the polymorphic molecular markers in VRM (Gg) 1 X TNAU RED cross by using 118 markers from different beans such as five primers from mungbean, seven from rice bean and 106 from azuki bean. From all these 118 markers, four markers from mungbean and forty-two markers from azuki bean have showed 54.07% and 39.62% polymorphism respectively. In this research, one species-specific SCAR molecular marker (400bp) based on RAPD marker was identified by designing primers in rice bean (Sudha *et al.*, 2013).

RAPD molecular markers are effortlessly and quickly detectable than RFLP molecular markers and can be converted into more reliable SCAR markers (Welsh and McClelland, 1990; Williams *et al.*, 1990). These markers could be effectively used for tagging resistance genes in mungbean against MYMV disease (Selvi *et al.*, 2006; Karthikeyan *et al.*, 2012). These markers were also reported in urdbean and mungbean (Maiti *et al.*, 2011). The investigation was conducted for identifying RAPD markers in *Vigna radiata* linked with resistance against MYMV. The marker was identified by applying bulk-segregant analysis in 93 recombinant inbred lines of F5 generation. Results showed that thirty-nine inbred lines were resistant to MYMV, eighteen lines were moderately resistant, twenty-seven lines were moderately susceptible, eight inbred lines were susceptible and only one inbred line was highly susceptible. Inbred lines which were highly susceptible and resistant from F8 generation were used for segregation analysis.

Total of twenty primers were used for RAPD marker identification in mungbean. From these twenty primers, just ten primers indicated genetic polymorphism between parental genotypes China mung and genotype BL 849. These ten primers; A-03, A-04 A-06, A-09, OPA-03, OPB 7, OPC-08, OPA-09, UBC-391 and UBC 499 were used for segregant analysis. From these ten primers, only one primer (UBC 499) revealed polymorphism between the bulks and the parents. This primer amplified a particular band of molecular size 700 bp. This amplification was present in resistant bulk and resistant BL 849 parental genotype but absent in susceptible bulk and China mung parental genotype. Primer also amplified a band of 700 bp in resistant bulk, parental resistant genotype (BL 849) and in nine individuals of F5 inbred lines. This indicated that UNC 499 primer was also associated with

resistance against MYMV disease. Another band with molecular size of 700 bp was amplified by primer in BL 489 genotype. These markers could be used for generating resistant genotypes of mungbean. For better reproducibility and reliability, UBC 49 marker can be changed into SCAR marker. Now this SCAR marker can be used for selecting resistant varieties against MYMV (Holeyachi and Savithramma, 2013).

Marker assisted selection (MAS) had great contribution in producing resistant varieties of mungbean with considerable success. Disease-linked molecular markers are becoming reliable and reachable for generating resistant germplasms (Maiti *et al.*, 2011; Chen *et al.*, 2013). Bulk segregant analysis is extremely applicable approach for identifying genetic markers linked to a specific trait and located at particular loci on chromosomes (Michelmore *et al.*, 1991; Collard *et al.*, 2005). The current research was conducted for detecting the molecular marker linked to MYMV disease in green gram using BSA approach. This was necessary for implicating molecular breeding techniques in developing resistant varieties of green gram.

Bulk Segregant Analysis (BSA) based on RAPD molecular marker approach was employed for analyzing the individuals of F2:3 generation from cross between PusaRatna, a susceptible variety and Meha, a resistant variety. The analysis was conducted for detaching MYMV-linked RAPD marker in mungbean plants. Inclusively, 35 genotypes of mungbean were screened against MYMV before crossing for detecting susceptible and resistant varieties. Among these 35 genotypes, two were highly resistant, eight genotypes were resistant, five were moderately resistant, nine were moderately susceptible, seven were susceptible and four genotypes were highly susceptible. Results of individuals in F2:3 population showed resistant, moderately resistant, susceptible, moderately susceptible and highly susceptible varieties. From these varieties, six highly susceptible and resistant varieties were selected for bulk analysis to identify molecular marker.

A total of thirty-eight RAPD primers were used for screening PusaRatna (MYMV-resistant parent) and Meha (MYMV-susceptible parent). Among these thirty-eight primers, only two primers (OPP 07 and OPS 07) showed genetic polymorphism among the parental genotypes. The OPP 07 primer amplified a particular band with size of 895 bp in resistant bulks and resistant parents. But there was no amplification by OPP 07 primer in susceptible bulks and parents because this specific band was absent in both genotypes. Co-segregation analysis was conducted to confirm the amplification by OPP 07 primer in susceptible and resistant bulk, individuals of F2:3 population and their parents. This specific band of 895 bp designated as molecular marker OPP 07₈₉₅ was found in resistant parents. This marker was also present in resistant bulks and six individuals of F2:3 generation but absent in susceptible genotypes, their bulks

and parents. So, it is confirmed that OPP 07₈₉₅RAPD marker was associated with resistance genes against MYMV in mungbean. The OPP 07₈₉₅marker can be utilized for detecting MYMV-linked QTLs. This MYMV-linked OPP 07₈₉₅RAPD marker could be converted into the SCAR marker for enhancing MAS effectiveness (Dharajiya and Ravindrababu, 2019). Protein markers are also helpful in differentiating resistant and susceptible genotypes but protein markers are not preferred over PCR-based markers because of their less yielding polymorphism capacity. A MYMV-linked protein marker was identified in mungbean (Pattnaik and Kole, 2002). The present research was conducted to identify PCR-based RAPD marker linked to MYMV in cross between ML 267 × CO 4 mungbean genotypes. Bulk segregant analysis was applied to identify MYMV-linked RAPD marker in resistant lines. Totally, 149 random RAPD primers (size of 10 nucleotides) were used for detecting RAPD marker in susceptible, resistant bulks of F₂ generation and their parents. About 90% from these primers showed amplifications. Out of these 149 primers, forty-one primers amplified a particular band in resistant genotypes but not in susceptible genotypes. From these forty-one primers, only three primers which were OPAK 19, OPT 16 and OPS 7 amplified specific bands of 400 bp, 564 bp and 900 bp respectively. These particular bands were supposed to be molecular markers; OPAK 19₄₀₀, OPT 16₅₆₄ and OPS 7₉₀₀. These putative molecular markers were present in resistant bulk and resistant parents but absent in susceptible bulk and parental genotypes. DNA samples from bulks were amplified with the OPS 7₉₀₀putative marker and that amplification showed polymorphism in six susceptible and eight resistant mungbean plants. This amplification confirmed the presence of OPS 7₉₀₀in ML 267 (resistant genotype) and its association with MYMV. This RAPD marker was converted into SCAR markers for increasing efficiency, accuracy and reproducibility. SCAR markers-based MAS of resistant mungbean genotypes is a valuable approach against MYMV disease (Selvi *et al.*, 2006).

The investigation was carried out to identify the RAPD marker in black gram. A total of thirty-three random primers were used for amplification of the specific fragments (206 bands) in resistant and susceptible varieties of black gram. A primer, OPH-3, amplified a particular band in PU-31 (resistant variety of black gram against MYMV) but this band was absent in other black gram genotypes. This particular band referred as OPH-3 RAPD marker in resistant variety. This RAPD marker was changed into SCAR marker to obtain efficient results in producing resistant varieties of black gram against MYMV (Vishalakshi *et al.*, 2017).

The investigation was pursued to locate the specific DNA marker in the blackgram genome against MYMV disease. Overall, 130 random primers were examined to find out the resistant varieties among these blackgram lines. From these primers, a particular oligonucleotide primer known as OPQ-1₅₂₅ amplified a specific band in resistant lines of blackgram.

This amplification was not observed in susceptible varieties. This primer was designated as the RAPD marker, RAPD OPQ-1. This marker differentiated the susceptible lines of blackgram from the resistant genotypes. The YMV-linked RAPD marker was clone and then sequenced for its conversion into SCAR marker. After sequencing, the end nucleotide sequences of OPQ-1 marker were exploited to design SCAR (20f/r) primer based on allele specificity. This primer amplified a particular region of 532 bp in the resistant genotypes that assigned as SCAR 20F/R marker (Prasanthi *et al.*, 2013).

This marker was then used to screen the forty-five lines of blackgram together with PU-19 and PU-31 varieties. Results showed that among these lines, nineteen lines were resistant, twelve lines were moderately resistant and the remaining lines were susceptible to YMV disease in blackgram. The YMV-linked SCAR primer amplified the resistance genes at 532 bp in all nineteen resistant lines. This particular band was absent in all susceptible varieties hence there was no amplification by SCAR (20f/r) primer in these lines. The amplification of resistance-linked marker confirmed that this marker is associated with YMV disease in blackgram. So, this marker can be used for identifying resistant varieties in other beans like mungbean, ricebean, azukibean using BSA approach. SCAR-marker based selection of resistant varieties from susceptible is extremely efficient comparing with RAPD-based selection because of its high reproducibility. So, this marker conversion is a helpful tool for successful bulk segregant analysis (Prasanthi *et al.*, 2013).

Plants can respond to pathogen infections by their own defense mechanism. This mechanism is associated with the resistance genes against a particular disease such as YMV diseases. Resistance gene analog (RGA) is a large group of resistance genes in plants species. These R-genes have conserved sequences that are helpful in predicting the functions of these genes. These genes confer resistance to plants against pathogens by producing R proteins. These proteins then activate defense mechanism against pathogens. RGAs from different plants can be obtained by using sequenced genome through various bioinformatics tools (Dilbirli *et al.*, 2003; Arya *et al.*, 2014). In recent years, whole genome of about fifty plants has been constructed and sequenced but a very few R-genes from this genome have been identified (Goodstein *et al.*, 2012; Monaco *et al.*, 2014; Nordberg *et al.*, 2014). They are irregularly distributed throughout the genome. These R-genes are helpful in generating resistant varieties against a number of pathogens. R-genes provide source for producing molecular markers and QTL linkage groups. These genes are important for breeding of resistant plants.

RGA have been identified in many monocot and dicot plant species. Among dicots, RGAs are present in Arabidopsis (Shiu *et al.*, 2004; Fritz-laylin *et al.*, 2005; Yu *et al.*, 2014), grape (Yang *et al.*, 2008; Yu *et al.*, 2014), papaya (Ming *et*

al., 2008), cucumber (Yang *et al.*, 2013), potato (Lozano *et al.*, 2012), *Medicago*, cabbage (Yu *et al.*, 2014), cotton (Chen *et al.*, 2015), strawberry (Li *et al.*, 2013), and apple (Arya *et al.*, 2014). Among monocots, these resistance genes are present in rice (Zhou *et al.*, 2004), maize (Cheng *et al.*, 2012), sorghum (Cheng *et al.*, 2010; Mace *et al.*, 2014), barley (Gu *et al.*, 2015) and in many pulses such as green gram, ricebean (Basak *et al.*, 2004).

RGA proteins are similar in structure and have many conserved domains such as NB domain (nucleotide binding site), coiled-coil domain (CC), transmembrane (TM), LRR domain (leucine rich repeats site), leucine zipper and toll receptor domain (Martin *et al.*, 2003; Monosi *et al.*, 2004; Van Ooijen *et al.*, 2007; Sanseverino *et al.*, 2010). Primers are designed based on these protein domains for isolating RGAs. In past years, RGAs have been isolated from sugarcane, maize, oilseed rape, cotton and other several plants. Resistance gene linked to a specific trait are cloned, sequenced, and then designed in to particular disease linked-RGA markers in plants (McIntyre *et al.*, 2005; Saal and struss, 2005; Xiao *et al.*, 2006; Gao *et al.*, 2010; Azhar *et al.*, 2011; Harris-Shultz *et al.*, 2012).

Molecular marker-based selection is intensively, efficiently and effectively used for studying the inheritance pattern of particular disease-linked genes (Peleman and van der Voort, 2003). Presence of resistance genes associated with resistance against YMV can be useful for distinguishing resistant varieties. Resistance-based molecular markers have been used to screen wheat population based on resistant genes, bunt resistance and leaf rust resistance gene (Scachermayr *et al.*, 1994; Demeke *et al.*, 1996). Resistance gene analog-based markers are the recently used marker comparing with other markers for screening R-genes (Chen *et al.*, 1998). These RGA markers are generated from the known motifs of the R-genes (Kanazin *et al.*, 1996; Huang and Gill, 2001; Yan *et al.*, 2003). In 1984, a resistant *Vigna mungo* plant was recognized in susceptible varieties (T9) of *V. mungo*. After that six resistant varieties were generated by self- crossing of these resistant plants.

In recent investigations, YMV-tolerant lines were crossed with T-9 *V. mungo* variety. After the crossing, F1 generation was produced. F2 and F3 generations were also raised by crossing between tolerant and T-9 plants. Field analysis of these generations indicated that a single recessive gene can control YMV infection. This was achieved by F2 population based on segregant analysis with phenotypic ratio of 3 (susceptible):1 (tolerant). Segregation analysis of F3 generation confirmed the presence of this gene. Only one primer, from total of twenty-four primers showed polymorphism among the resistant parents. This primer amplified a band of 445 bp in the tolerant plants. These amplicons were cloned and sequenced for designing RGA marker. This sequence was then designated as VMYR1, present in homozygous tolerant lines but absent in

heterozygous parents. The sequence of amino acid for this RGA marker was resembled to NB-ARC part of resistance genes against several diseases in crop plants. This was the first discovered RGA marker linked To YMV in black gram via segregating individuals. This will be helpful in MAS of tolerant plants. This tightly YMV-linked RGA marker can be potentially used for breeding of many other plants like mungbean, urbean etc. MAS will also be valuable for increasing productivity, yield and many other desirable traits. This can be achieved in relatively less duration comparing with other means of selection like phenotypic selection (Basak *et al.*, 2005).

Resistance genes are distinct genes distributed through the genome against a certain disease. Development of the markers linked to these resistance genes can be carried out by exploitation of known nucleotide information of these gene in other crop plants. In previous experiments, seven resistant lines of blackgram (VM1, VM2, VM3, VM4, VM5, VM6, and VM7) were produced from a susceptible T9 plant that was generated by selective breeding (Kundagrami *et al.*, 2009). From that research, a VMYR1 marker was detected against MYMIV but due to its dominant character it could not discriminate susceptible variety that was heterozygous from the resistant lines (Basak *et al.*, 2004). The research was conducted in an attempt to develop molecular markers that are specifically associated with the Mungbean Yellow Mosaic India (MYMIV). The germplasms of mungbean and urbean were used for genotyping of resistance gene linked to MYMIV. Studies indicated that resistance genes, related to viral disease, possess NBS-leucine rich repeats domain. This domain is conserved in most of the resistance genes of plants (Kang *et al.*, 2005). From these known regions, primers were designed for isolating RGAs in number of plants such as pulses, cereals, cotton and sugar beet (Kanazin *et al.*, 1996). RGA primers were isolated from the *Fabaceae* family to identify polymorphism of contrasting genotypes (Basak *et al.*, 2004; Pal *et al.*, 2007). These RGA primers amplified a molecular band in the resistant varieties and not in the susceptible one that led to identification of two loci linked to resistance against MYMIV. Susceptible genotypes were more vulnerable to this disease. These two loci, CYR1 and YR4 were named as RGA-linked markers but YR4 was partially linked to the MYMIV resistance and CYR1 was strongly linked to that virus in the resistant germplasms. Co-segregant studies of F2 and F3 populations have been confirmed the presence of this marker in the resistant germplasm of urbean. These markers could be employed for genotyping of both the germplasms from mungbean and urbean by using multiplex PCR. This genotyping approach will be easy and time-saving. From CYR1 marker, heterozygous susceptible plants can also be distinguished because of its complete association with MYMIV disease (Maiti *et al.*, 2011).

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